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Facilitating production of a protein for analyzing, designing and/or modifying an agent that can interact with a viral F protein, comprises expressing a nucleic acid optimized for expression of the protein, using a eukaryotic cell - vector-mediated gene transfer and expression in host cell for recombinant vaccine and gene therapy

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PATENT ASSIGNEE: BIOTA SCI MANAGEMENT PTY LTD 2002

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PRIORITY APPLIC. NO.: US 252767 APPLIC. DATE: 20001122

NATIONAL APPLIC. NO.: WO 2001AU1517 APPLIC. DATE: 20011122

LANGUAGE: English

ABSTRACT: DERWENT ABSTRACT: NOVELTY - Facilitating (M1) production of a protein or its derivative (I) from a negative sense single stranded RNA virus, by expressing a nucleic acid molecule (NAM) encoding (I) in a host cell, where the nucleotide sequence of NAM is optimized for expression by a eukaryotic cell. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an optimized NAM or its derivative, equivalent, analog or mimetic (II); (2) a protein molecule (I) encoded (II); (3) regulating (M2) the functional activity of a viral F protein, where the protein in its non-fully functional form comprises an F2 portion linked, bound or otherwise associated with an F1 portion, and where the F2 portion comprises an intervening peptide sequence, by modulating cleavage of the intervening peptide sequence where excision of a portion of the intervening sequence from the non-fully functional form of the protein up-regulates F protein functional activity; (4) detecting (M3) an agent capable of regulating the functional activity of a viral F protein or its derivative by contacting an eukaryotic cell expressing an optimized NAM with a putative modulatory agent and detecting an altered expression phenotype and/or functional activity; (5) an agent (III) capable of interacting with a viral F protein and modulating a functional activity associated with the viral protein; (6) a viral F protein variant (IV) comprising a mutation in the intervening peptide sequence, where the variant exhibits modulated functional activity relative to wild-type F protein or its derivative, homolog, analog, chemical equivalent or mimetic; (7) a recombinant viral construct (RVC) comprising NAM, where the recombinant viral construct is a effective in inducing, enhancing or otherwise stimulating an immune response to the F protein; and (8) a vaccine comprising RVC. BIOTECHNOLOGY - Preferred Method: In M1, the virus is from family Paramyxoviridae, and sub-family Pneumovirinae, and more preferably the virus is respiratory syncytial virus (RSV). The protein directly or indirectly facilitates fusion of any one or more viral components with any one or more host cells components, where (I) is a F protein or its derivative, which is the Fsol fragment, or is an N, P or SH protein or its derivative. The eukaryotic host cell is preferably a mammalian cell which is a 293 cell, or a Chinese Hamster Ovary Cell, where the optimization is: (a) a codon optimization which comprises modification of an A and/or T comprising codon to express G and C, respectively and the splice site deletion comprises deletion of an RNA splice site; and/or (b) a nucleotide splice site deletion, where the optimized pro

QR189.U7, Adams

10875881 97227577 PMID: 9139483

Immunization of mice with poliovirus replicons expressing the C-fragment of tetanus toxin protects against lethal challenge with tetanus toxin.

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Vaccine (ENGLAND) Feb 1997, 15 (3) p257-64, ISSN 0264-410X Journal Code: 8406899

Contract/Grant No.: AI25005; AI; NIAID; AI27767; AI; NIAID; AI28147; AI; NIAID

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

In this study, we describe the construction of poliovirus genomes or "replicons" which contain the C fragment gene of tetanus toxin substituted for the poliovirus P1 capsid. Upon transfection of replicon RNA into cells, we immunoprecipitated a protein corresponding to the C-fragment of tetanus toxin using tetanus-specific antibodies. Using a recombinant vaccinia virus expressing poliovirus P1 capsid protein (VV-P1) to provide P1 protein, the replicon RNA was encapsidated; stocks of the replicons were generated by passage with VV-P1. The immunogenicity of the replicons was determined by immunization of transgenic mice which are susceptible to poliovirus. A serum antibody response to poliovirus and tetanus toxoid was detected in all of the immunized mice. Protection against a lethal dose of tetanus toxin generally correlated with the levels of serum anti-tetanus antibodies. To address whether pre-existing antibodies to poliovirus limit the effectiveness of the replicon as a vaccine vector, mice were first immunized with the inactivated poliovirus vaccine followed by immunization with the replicons expressing C-fragment protein. Anti-tetanus antibodies were detected in these mice after a single administration of the replicon; these antibodies conferred protection upon challenge with tetanus toxin. These results demonstrate the potential use of poliovirus replicons encoding foreign proteins to induce a protective antibody response, even in the presence of pre-existing antibodies to poliovirus.

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15/7/13 (Item 13 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

Micro

06168105 89183595 PMID: 2928109
Codon usage and secondary structure of MS2 phage RNA.
Bulmer M
Department of Statistics, Oxford, UK.
Nucleic acids research (ENGLAND) Mar 11 1989, 17 (5) p1839-43,
ISSN 0305-1048 Journal Code: 0411011
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
MS2 is an RNA bacteriophage (3569 bases). The secondary structure of the RNA has been determined, and is known to play an important role in regulating translation. Paired regions of the genome have a higher G+C content than unpaired regions. It has been suggested that this reflects selection for high G+C content to encourage pairing, but a re-analysis of the data together with computer simulation suggest that it is an automatic consequence in any RNA sequence of the way it folds up to minimise its free energy. It has also been suggested that the three registers in which pairing can occur in a coding region are used differentially to optimise the use of the redundancy of the genetic code, but re-analysis of the data shows only weak statistical support for this hypothesis.
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15/7/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11157186 98033196 PMID: 9367373

Dinucleotide and stop codon frequencies in single-stranded RNA viruses.

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Journal of general virology (ENGLAND) Nov 1997, 78 (Pt 11) p2859-70, ISSN 0022-1317 Journal Code: 0077340

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

To identify potential selection pressures which lead to RNA sequence conservation, we examined the occurrence rates of dinucleotides in 64 single-stranded RNA virus genomes. These viruses may offer a particular insight into these pressures since their RNA-dependent RNA polymerases lack proofreading capability. This potentiates introduction of mutations into their genomes, yet unidentified selection processes conserve the genomes to a large degree. We report a strong inverse correlation between the C+G content and the occurrence of the CpG dinucleotide (r=0.71) in the RNA virus genomes, in contrast to earlier reports (Karlin et al., 1994, Journal of Virology 68, 2889-2897). We also detected significant suppression of UpA, correlating inversely with genomic U+A content. These suppressions are coupled with over-representation of the complementary pair of dinucleotides, CpA and UpG. In addition, we highlight the fact that odds ratios for dinucleotides are not independent variables, a situation apparently not widely appreciated in the literature. This led us to view the over-representation of CpA and UpG as a consequential outcome of UpA and CpG suppression in the virus genomes. Potential factors influencing these disturbances are discussed. In addition, higher than random incidence was observed for 'out-of-frame' stop codons in the viral RNA genomes, with some preferences for individual codons being exhibited by certain virus groups. The UAG codon appeared more common in the +1 frame, the UGA in the -1 frame.

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11/7/16 (Item 16 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05929957 88284374 PMID: 2456256

Alteration of amino-terminal codons of human granulocyte-colony-stimulating factor increases expression levels and allows efficient processing by methionine aminopeptidase in Escherichia coli.

Devlin P E; Drummond R J; Toy P; Mark D F; Watt K W; Devlin J J
Department of Molecular Biology, Cetus Corporation, Emeryville, CA 94608.

Gene (NETHERLANDS) May 15 1988, 65 (1) p13-22, ISSN 0378-1119 Journal Code: 7706761
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

We have improved the expression of recombinant human granulocyte-colony-stimulating factor (G-CSF), produced by either pL or trpP expression vectors in Escherichia coli, by altering the sequence at the 5' end of the G-CSF-coding region. Initial attempts to express G-CSF resulted in neither detectable G-CSF mRNA nor protein in the trpP system, and only G-CSF mRNA was detectable in the pL system. We modified both expression vectors to decrease the G + C content of the 5' end of the coding region without altering the predicted amino acid sequence. This resulted in expression of detectable G-CSF mRNA and protein in both systems. Expression reached 17% and 6.5% of the total soluble cellular protein in the pL and trpP expression systems, respectively. The N-terminal sequence of the recombinant G-CSF from the pL system was Met-Thr-Pro-Leu-Gly-Pro-. G-CSF isolated from several human cell lines (including the LD-1 cell line reported here), does not have an N-terminal methionyl residue. Deletion of the threonine codon at the beginning of the coding region for the mature G-CSF resulted in efficient removal of the N-terminal methionine residue during expression in E. coli.

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